Sara E. Monaco
Lisa A. Teot *Editors*

Pediatric Cytopathology

A Practical Guide

Springer

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Sara E. Monaco, MD
To my parents, husband, and children Eddie, Julia,
and Nicholas for their endless love and support.

Lisa A. Teot, MD
To my parents, for their love and for always believing in me,
and to Mark, for his love, quiet support,
and calm understanding.

Foreword

Pediatric cytopathology is so much more than just another area of our expanding specialty. It is as heavily dominated by tumor diagnosis as other branches, but, involving children and teenagers as it does, this arena evokes a heightened sense of crisis to parents, patients, and ourselves as practitioners. Most pediatric tumors are potentially lethal if left untreated, and many of them present acutely. At the same time, prospects of cure or at least significant remission with appropriate therapy are generally far greater than encountered with most common tumors of adults. This is therefore an area wide open for early, quick, and accurate cytodiagnosis to enable rapid determination of tumor type, with molecular and genetic characteristics. At the same time, exclusion of malignancy by confident demonstration of benign lesions, many of them inflammatory, enables rapid institution of appropriate treatment and gives relief to all that neoplasia is not involved.

With this in mind, it may appear that there would be a plethora of texts on pediatric cytopathology. This is not the case; pediatric tumors are generally uncommon in the population at large; few generalists are able to develop daily expertise in all but a few tumor types. Into this significant gap comes the awaited text by Dr. Sara Monaco, of the Children's Hospital, University of Pittsburgh, and Dr. Lisa Teot of Boston Children's Hospital. Both authors draw on a wealth of experience and involvement in pediatric cytopathology. It is clear that they both embody enviable expertise, gained over time at their large academic institutions with active pediatric programs. Not only are they immersed in microscopy but also in active collection of cytologic material from young and apprehensive patients, often accompanied by parents, the latter experiencing one of the most grave circumstances a child's parent may know. This is a fraught environment many pathologists never encounter.

This major text will be a standard and a benchmark in cytopathology. It will serve all practitioners in this most necessary of specialty areas. It combines basic experience with up-to-date concepts in an accessible and readable text, generously illustrated with clear color images. The chapters are well ordered and fully comprehensive, incorporating not only microscopic diagnoses but immunochemical and molecular data as well.

A significant feature of this book is the inclusion of numerous informative tables, enabling the practitioner to readily view comprehensive differential diagnoses in an easily accessible format. The authors have created these new clear and concise aids which perfectly complement both the text and the images. With this format, the book presents itself both as a reliable reference

and as a ready desk handbook to be used at the microscope when viewing unusual and challenging entities.

This new text is an essential addition to every cytopathology departmental library and is geared for use by cytopathologists and cytotechnologists alike. Moreover, for surgical pathologists tasked with looking at rare pediatric tumors, the book enables knowledgeable viewing of unusual entities. Sara Monaco and Lisa Teot have given us an important and much anticipated volume which will be used for years to come.

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Preface

Pediatric cytopathology is a challenging area of anatomic pathology. This is due in part to the limited use of cytology as a diagnostic modality in the pediatric age group, particularly in North America, and in part to the different spectra of diseases encountered in this population as compared to adults. With the increased use of minimally invasive techniques to obtain diagnostic specimens from pediatric patients, the need has evolved for a current reference focused on the cytopathology of entities that are either unique to or more common in children and adolescents.

The aim of this book is to provide an up-to-date reference focusing on the cytomorphology of the common and uncommon entities encountered in the pediatric population, richly illustrated with full-color, high-resolution images. Challenges and diagnostic pitfalls are also highlighted. Each chapter includes tables which summarize key points, as well as features used to resolve the differential diagnosis.

This book is intended to be a concise yet comprehensive reference for practicing pathologists and cytopathologists, pediatric pathology fellows, cytopathology fellows, residents in anatomic pathology, pediatricians, and pediatric subspecialists. We hope that it will serve as a practical and useful guide and enhance the skills of those involved in the practice of pediatric cytopathology.

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Introduction

1

Sara E. Monaco and Lisa A. Teot

1.1 Introduction

Fine needle aspiration (FNA) is a reliable, minimally invasive, cost effective technique for obtaining samples from superficial and deep mass lesions for pathologic evaluation. Despite these advantages, physicians in the USA have been slow to embrace FNA as a primary diagnostic modality in the pediatric population. Obstacles to the acceptance and use of FNA include diagnostic challenges posed by the overall rarity and spectrum of tumors seen in children and adolescents, the experience and biases of clinicians and pathologists, and practical and technical considerations. Cytopathologists who are experienced in the performance and interpretation of FNAs may have limited familiarity with the spectrum and morphologic appearances of tumors seen in the pediatric population. Conversely, pediatric pathologists who are familiar with the histologic features and differential diagnosis of tumors encountered in children and adolescents often have little experience performing and/or interpreting FNAs. Likewise, clinicians who have extensive experience performing endoscopic or endobronchial ultrasound guided FNAs may have little experience with endoscopy or bronchoscopy of pediatric patients, and vice versa. These factors can impact the quality of the specimen and/or interpretation and lead to the need for a second procedure in order to arrive at a definitive diagnosis, thereby limiting the value of FNA as a diagnostic modality. Practical considerations include the cognitive and emotional maturity of the child or adolescent, and the need for immobilization, sedation, or anesthesia. Alone or in combination, these and other challenges and limitations have contributed to reluctance on the part of both pathologists and clinicians to promote the use of FNA as a primary diagnostic modality in the pediatric population. In contrast, exfoliative cytology is routinely used in the evaluation of cerebrospinal fluid and respiratory tract specimens from children and adolescents, and smears and crush preparations are standard methods for intraoperative assessment of pediatric central nervous system lesions.

1.2 Spectrum of Practice

The use of FNA as a primary diagnostic modality in the pediatric population varies with geographic location, practice setting, and clinical environment (Table 1.1) [1]. With respect to geographic

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Table 1.1 Factors influencing the use of FNA in the pediatric population

• Geographic location (resource-limited, resource-rich)
• Type of practice (academic, community)
• Presence of a free-standing pediatric hospital
• Organization of practice (subspecialty based or general pathology)
• Clinical environment (experience with and acceptance of FNA, referral patterns)
• Availability of physicians trained in performance and interpretation of fine needle aspiration
• Sensitivity and performance of fine needle aspiration (diagnostic vs. inadequate or non-diagnostic specimens, definitive or narrowed diagnoses that effectively guide management vs. nonspecific diagnoses/need for additional biopsy)

location, 86 % of the world's pediatric population lives in resource-limited or developing countries where malignancies in children and adolescents comprise a greater percent of all cancers and have a higher mortality rate than in the USA and Europe [2]. In countries where access to medical care, diagnostic imaging, and more invasive procedures such as core or excisional biopsy is limited, FNA is routinely used for the primary evaluation of suspected malignancies in the pediatric population and has proven to be an accurate diagnostic tool [3, 4]. In contrast, FNA is rarely used for the primary diagnosis of pediatric malignancies in the USA where there is widespread access to more invasive diagnostic modalities and where risk stratification and treatment are often based on histologic diagnosis.

Within the USA and other resource-rich countries, the volume of pediatric FNAs can also vary greatly in different practice settings. Clinicians who have had positive experiences with FNA as a diagnostic modality are more likely to consider referring patients for FNA or to recommend the use of FNA to their colleagues, than those who have had negative experiences. Acquisition of an adequate specimen, appropriate triage, and diagnostic expertise are all required for providing a high quality FNA service. Adequate samples can be obtained by pathologists, interventional radiologists, and/or clinicians with appropriate training and expertise in performing FNAs. However, within a given institution, the type(s) and availability of qualified physicians impacts whether FNAs are performed in inpatient and/or outpatient settings, or not at all, and whether the lesions sampled are superficial and/or deep. Appropriate triage of the specimen is essential when ancillary studies are needed for a definitive or narrowed differential diagnosis. Rapid on site evaluation (ROSE) not only allows assessment of adequacy, but also guides appropriate triage of the specimen. However, ROSE can be time consuming and is deemed economically impractical in some practice settings. The availability of pathologists and/or cytotechnologists to perform ROSE can have a significant impact on whether the procedure results in a definitive or narrowed differential diagnosis and thus, on the use of FNA rather than a more invasive core or open biopsy for the primary evaluation of a mass lesion in a child or adolescent. Finally, the expertise required for accurate cytologic diagnosis of pediatric lesions is more likely to be found in settings with subspecialty-trained cytopathologists and pediatric pathologists, and can have a positive impact on the use of FNA. In general, the key elements for the acceptance and successful use of FNA as a diagnostic modality in the pediatric population are more likely to be found in an academic institution than in a community hospital.

Geographic location and practice setting also influence the type and pathologic spectrum of pediatric lesions evaluated by FNA. In resource-limited countries, malignancies comprise the majority of lesions diagnosed by FNA [4], while in resource-rich countries benign processes predominate [1]. Moreover, in resource-limited countries, a greater proportion of malignancies diagnosed by FNA are primary and/or deep-seated tumors than in resource-rich countries. In the USA, primary cytologic diagnosis of malignancies is rare; rather, FNA is primarily used for the evaluation of superficial masses, the majority of which are benign and located in the head and neck [1]. It is important to note that this

pattern is observed even in institutions with robust pediatric FNA services and, in part, reflects the fact that Children's Oncology Group therapeutic protocols are based on histologic diagnosis and associated biologic studies require frozen or formalin-fixed tissue.

1.3 Diagnostic Considerations

Mass lesions in children and adolescents raise different diagnostic considerations than those in adults. In the pediatric population, malignancies are rare and comprised predominantly of hematolymphoid and central nervous system neoplasms. In contrast, in the adult population, cancer is common and epithelial neoplasms account for the vast majority of malignancies. Unlike in adults, small changes in age can significantly alter the differential diagnostic considerations in the pediatric population [5]. Table 1.2 lists the three most common types of malignancies in different age groups, and illustrates the changes observed with small increments of age. The types of tumors seen in a given anatomic site also vary with age. In the kidney, for example, mesoblastic nephroma is usually diagnosed in the first 3 months of life, whereas Wilms tumor is most common in children under 5 years of age, and renal cell carcinoma primarily affects adolescents. A variety of genetic syndromes are also associated with increased risk of developing certain pediatric tumors, as illustrated by the increased risk of Wilms tumor in children with Beckwith–Wiedemann, WAGR (Wilms tumor, aniridia, genitourinary malformation, and mental retardation), and Denys–Drash syndromes. Awareness of the types of tumors that arise at different ages in various anatomic locations and of the associations between genetic syndromes and certain types of tumors is important for accurate cytologic diagnosis of pediatric mass lesions.

Table 1.2 Cancer incidence by age group in children based on data from the Automated Childhood Cancer Information System ([5], adopted from ref. [2])

Age group	Tumor category
Infants (less than 1 y.o.)	#1: Sympathetic nervous system tumors
	#2: Leukemia
	#3: CNS tumors
Young children (1–4 y.o.)	#1: Leukemias
	#2: CNS tumors
	#3: Renal tumors
School-age children (5–9 y.o.)	#1: CNS tumors
	#2: Leukemias
	#3: Lymphomas
Older school-age children or young adolescents (10–14 y.o.)	#1: Lymphomas
	#2: Leukemias
	#3: CNS tumors
Older adolescents (15–19 y.o.)	#1: Lymphomas
	#2: Carcinomas
	#3: Germ cell tumors

In addition to these considerations, morphologic similarities between pediatric malignancies can pose diagnostic challenges. Many of the most common pediatric malignancies are small round blue cell tumors, while a variety of benign and malignant neoplasms have spindle cell morphology. Ancillary studies, such as immunoperoxidase stains, flow cytometry, fluorescence in situ hybridization, and/or other molecular tests, are usually required for definitive diagnosis, thereby making appropriate triage of these specimens critical. Treatments for many of these tumors vary considerably and thus, an accurate, specific diagnosis is essential. In contrast, for benign and low-grade spindle cell neoplasms for which treatment consists of surgical excision and for non-rhabdomyosarcomatous high-grade spindle cell sarcomas for which chemotherapy is the same, it may be sufficient to exclude certain entities and provide a narrowed differential diagnosis.

1.4 Conclusion

This book will provide a practical reference for pathologists evaluating cytologic specimens from pediatric patients. It is organized in an organ-based manner to address the spectrum of lesions seen in this population, and highlights important ancillary studies and differential diagnostic considerations.

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Fine Needle Aspiration in Pediatric Patients: Approach and Technique

2

Sara E. Monaco and Lisa A. Teot

2.1 Introduction

The statement "children are not just small adults" applies not only to clinical medicine, but also to pathology, as evidenced by formal recognition of pediatric pathology as a subspecialty. Successful use of fine needle aspiration (FNA) for the pathological evaluation of pediatric lesions poses special challenges and requires consideration of the patient's level of cognitive and emotional maturity, awareness of the diseases that occur in this population, and knowledge of the cytological features of those entities.

In children and adolescents, as in adults, FNA has the advantage of being a minimally invasive technique for obtaining diagnostic material from mass lesions. In experienced hands and in the appropriate clinical context, FNA has a sensitivity of approximately 97–98 % and a specificity of 93–97 % [1, 2]. In the pediatric population, use of FNA is particularly beneficial for evaluation of superficial lesions, many of which are reactive or infectious in origin. In this setting, FNA can confirm the benignity of the lesion and in some cases, provide a specific diagnosis through the use of ancillary studies, while avoiding the greater risks of core or open biopsy. For both benign and malignant lesions, on-site evaluation at the time of the procedure is important and can help to ensure adequacy, guide appropriate triage and, thereby, minimize the likelihood of a non-diagnostic specimen. When the FNA is performed by someone other than the pathologist, on-site evaluation also affords an opportunity to provide important feedback to the proceduralist and, for suboptimal specimens, may allow conversion to core biopsy, thereby averting the need for a second diagnostic procedure at a later time. While it is desirable to avoid the need for a repeat FNA or additional biopsy irrespective of the patient's age, subjecting a child or adolescent to a second procedure may be particularly burdensome to the patient and family in terms of emotional distress, the costs associated with time away from school, work, and caring for siblings and, in some cases, the need for sedation or anesthesia, none of which is trivial.

This chapter will highlight some of the key factors to consider when performing FNAs on pediatric patients.

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2.2 Pre-procedural Evaluation

The pathologist who performs an FNA serves as a consultant to the referring clinician and therefore, usually meets the patient and parent(s) or legal guardian for the first time at the time of the FNA. Ideally, the referring physician or physician extender communicates to the pathologist in writing his or her clinical suspicions and any pertinent history, physical findings, laboratory results, and/or imaging studies on which they are based, as well as any relevant pending studies. This can occur through a medical record to which the pathologist has access or through a letter or written request for consultation in cases in which the medical records are inaccessible to the pathologist. Oral communication initiated either by the clinician at the time of referral or by the pathologist when written communication from the referring clinician is lacking is an acceptable alternative, although less desirable due to the possibility of errors. The pathologist should review the patient's medical record when it is accessible, irrespective of any communication with the referring clinician. Ideally, information from the referring clinician and/or medical record provides the pathologist with important background data that may or may not be elicited at the time of FNA, but is not intended to replace communication between the pathologist and the patient and parent(s) or legal guardian. In addition to conveying important medical information, communication between the referring clinician and pathologist provides an opportunity to address key issues related to consent, such as who a minor child's legal guardian is and the need for an interpreter (see Sect. 2.3).

2.2.1 Clinical History

As noted above, the initial encounter between the pathologist performing an FNA and the child and parent(s) or legal guardian is usually at the time of the procedure. A successful interaction with the child and parent(s) or legal guardian requires appreciation of the child's level of apprehension, which may range from virtually absent to intense and is shaped by cognitive and emotional maturity, prior experiences with medical personnel and vaccinations, the degree to which the child has been prepared for the FNA, and his or her expectations around the procedure. Wearing street clothes rather than a white coat, and engaging the child in age appropriate conversation or other interactions prior to obtaining a clinical history and performing a physical examination can help to establish rapport with the child, as well as the parent(s) or legal guardian. At each stage of the encounter, it is important to include the child in the conversation at his or her cognitive level and talk with the child at his or her eye level, rather than simply talking about the child with the parent(s) or legal guardian. This is particularly important when the patient is a school age child or adolescent.

Clinical history is helpful for formulating a differential diagnosis and can provide important clues to the correct diagnosis. It is important to obtain a clinical history directly from the patient and/or parent(s) or legal guardian, rather than simply relying on information communicated by the referring clinician and/or contained in the medical record. Beyond helping to establish rapport with the patient and parent(s) or legal guardian, this allows the pathologist to validate the clinical history provided elsewhere and resolve any potentially important discrepancies or omissions. For example, when a child is referred with lymphadenopathy, there should be a discussion about exposure to cats or other animals, as well as any recent travel. The qualities of the lesion and how they have changed over time are also important. A mass lesion that has persisted and enlarged over a short period of time is more concerning for a malignancy than a lymph node that fluctuates in size over time. Results of laboratory tests, serologic studies, and microbiologic cultures can also be helpful. For example, in developed countries, many children and adolescents presenting with persistent lymphadenopathy will have had a tuberculin skin test, monospot test, Epstein–Barr virus (EBV) IgM and IgG titers, and *Bartonella* titers. Having the results of these tests can be very helpful when approaching the evaluation of these cases, although results may

not be available at the time of the procedure or may not have been conveyed to the parent(s) or legal guardian by the ordering clinician.

2.2.2 Physical Examination

After obtaining a history, a directed physical examination establishes the size of the lesion, its mobility (freely mobile or fixed), contour (ill- or well-defined), texture (soft, doughy/cystic, or firm), and any associated tenderness. This tactile contact with the patient not only allows the pathologist to guage the patient's level of anxiety about the procedure, but may also help the patient to feel more comfortable with the pathologist. When examining a school age child or adolescent, especially of the opposite gender, it is suggested that the pathologist be accompanied by a nurse or other health care assistant. Parents can be asked to leave or may stay, depending on the preference of the child.

2.3 Informed Consent

Prior to performing the FNA, informed consent is obtained. This includes an explanation of the procedure, its benefits, potential complications, and risks, including a non-diagnostic aspirate and the need for a second diagnostic procedure, and alternatives to FNA. The consent form is signed and dated by the physician and the patient's legal guardian, which in most cases is the parent. The consent form becomes part of the medical record. For children under the age of 18, who cannot legally consent to undergoing a procedure, it is imperative that the pathologist confirm prior to the appointment who will be accompanying the child and who the legal guardian is. If someone other than the legal guardian or parent will accompany the child, then it is important to obtain informed consent from the legal guardian or parent beforehand, usually by telephone with at least one witness. This occurs most often when a child who is in a foster home or other institution is accompanied by someone other than the legal guardian, but can also arise when a child is accompanied by a relative, such as a grandparent, who is not the legal guardian. A social worker or risk management personnel can usually help to determine and, if necessary, locate the legal guardian. However, this can take time and may delay or necessitate rescheduling of the procedure if not done beforehand. Of note, the consent laws of about 30 states and the District of Columbia give patients who are minors but are parents, married, or pregnant the legal capacity to consent for a procedure, while the remaining states have no explicit policy or law [3]. It is also recommended that, as part of the consent process, assent be obtained from school age children and adolescents to confirm that they are willing to undergo the FNA and to ease their anxiety. When obtaining consent and/or assent, it is important to be at eye level, to turn off all electronic devices that could be a distraction, to use basic language rather than medical terminology, and to make sure that the parent(s) or legal guardian and child understand what they have been told, are given the opportunity to ask questions and that their questions are answered to their satisfaction. When necessary, an interpreter employed by the facility in which the FNA is performed should be provided to ensure that the parent(s) or legal guardian and child understand the pathologist's explanations, have had their questions answered satisfactorily and are truly giving informed consent.

2.4 Equipment

The equipment required for the FNA procedure includes syringes, needles (22–27G), a syringe holder, such as the Cameco Syringe Pistol, glass slides, pencil or permanent marking pen, spray fixative or Coplin jar with 95 % alcohol, slide folders or plastic slide holders, staining solutions, sterile tubes, formalin-filled containers, alcohol wipes, gauze, band-aids, and personal protective equipment (Figs. 2.1 and 2.2). In the pediatric setting, it can be helpful to have colorful band-aids with patterns, superheroes, or other characters that children can identify with. These items can be stored in a labeled basket or cart for convenience

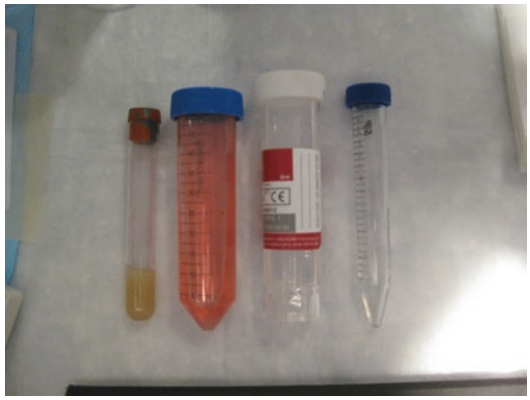

Fig. 2.1 Supplies for pediatric FNAs. Multiple supplies are needed during pediatric FNAs because of the variety of ancillary studies that may be required. It is usually helpful to have a variety of different containers (shown from *right* to *left*), including sterile tube for microbial cultures (*right*), liquid based cytology containers (e.g., Thin Prep™; *middle right*), container with fresh cold Roswell Park Memorial Institute (RPMI) media for flow cytometry (*middle left*), and tiger-top blood collection tubes (*left*) for tapping needles that have clotted material.

Fig. 2.3 An FNA basket utilized to carry materials to procedures. A crate or sturdy plastic tool box can be used to hold the materials needed for an FNA and allows the pathologist to be mobilized quickly to perform an FNA on a child in an outpatient clinic, operating room, or inpatient setting. An opaque container also maintains patient confidentiality when carrying materials back to the cytology laboratory after a procedure.

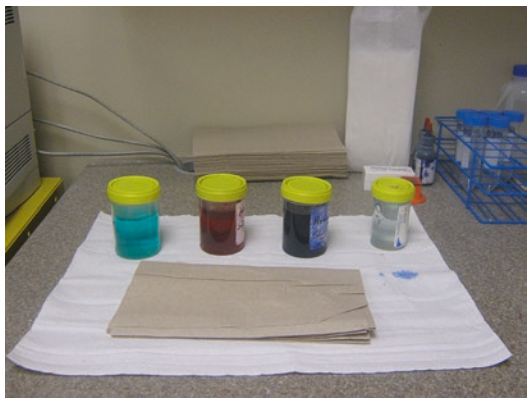

Fig. 2.2 Diff-Quik staining supplies. If on-site evaluation is performed, a rapid stain is necessary, such as a Romanowsky-type stain, like Diff-Quik. The staining takes less than 1 minute and is performed on air-dried smears. These slides can be examined without coverslipping.

(Figs. 2.3 and 2.4). If on-site evaluation and preliminary diagnosis are desired, then a microscope is also required, which can be placed on a mobile cart or in a permanent location in sites where FNAs are frequently performed (Fig. 2.4). A papoose or large blanket to wrap a child's extremities can also be very helpful to immobilize the non-sedated patient who is unable to cooperate (Fig. 2.5).

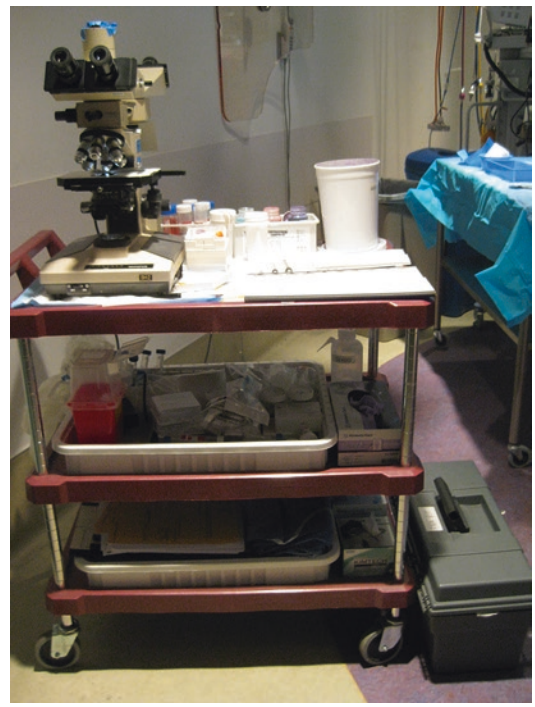

Fig. 2.4 FNA cart utilized for on-site evaluations. If on-site evaluation of an FNA is required, then an FNA cart stocked with a microscope and all necessary supplies is important.

Fig. 2.5 Papoose for immobilization of non-sedated pediatric patients. These immobilization devices allow the child to lie down on the flat board, while soft cloth arms are wrapped and secured around the child's arms and legs to prevent them from moving during the FNA procedure.

2.5 Fine Needle Aspiration Procedure

FNAs are performed by pathologists and other physicians in a variety of locations, including outpatient clinics, the operating room, at the bedside of hospitalized patients, and in the radiology suite. For non-palpable masses detected by imaging, CT or ultrasound (US) guidance should be used to perform the FNA. In addition to interventional radiologists, some pathologists are qualified to perform US-guided FNAs and may use portable ultrasound equipment in the clinic, operating room, or at the bedside. The techniques involved in US-guided FNA are beyond the scope of this discussion, which will be confined to FNA of palpable lesions. Prior to beginning the FNA, a "time out" is performed and documented to confirm the procedure, the patient's name and unique identifiers, and the location (anatomic site and laterality) of the FNA. This pause allows everyone to confirm that the correct procedure is performed on the correct patient and the correct lesion.

2.5.1 Palpation and Immobilization of the Lesion

The first steps in performing an FNA are palpation and immobilization of the lesion. Palpation is performed at the time of physical examination to investigate the size, mobility, contour and consistency of the mass, and presence or absence of associated tenderness. It is repeated prior to sampling primarily to confirm the location and accessibility of the lesion. Before proceeding with immobilization and sampling of the lesion, children who are developmentally unable to cooperate and are not under general anesthesia must be securely positioned with a nurse and/or parent helping to immobilize their arms and legs. If the child is strong or there are not enough people to assist with the procedure, then a papoose can be utilized to secure the child (Fig. 2.5). In some cases the FNA is performed under conscious sedation or general anesthesia at the request of the parent and/or discretion of the clinician. An ideal time to perform an FNA is when the child is undergoing general anesthesia for another procedure (e.g., FNA of an enlarged cervical lymph node during anesthesia for placement of myringotomy tubes) and can be optimally positioned with no movement; however, this is not an option in all cases. Once the patient is immobilized, the lesion itself can be immobilized with the fingers of the non-dominant hand, usually the index and middle fingers in order to reserve the thumb for stabilizing the needle and syringe holder. In young or anxious patients, topical anesthetic, such as 4 % topical lidocaine cream, can be applied prior to the procedure to decrease discomfort during the FNA and is typically tolerated better than subcutaneous injection of 1 % lidocaine with 1:100,000 epinephrine.